

the allergenic activity of this enzyme. A positive challenge response to 10 mg of uncooked α -amylase in a pharmaceutical worker who was exposed to powdered *Aspergillus* α -amylase was reported previously. In that case, the ingestion of bread was tolerated without any symptoms. The use of α -amylase as an additive has been authorized in France since 1983. The amount of α -amylase contained in bread is not negligible: French bread contains approximately 4 to 18 mg of the enzyme per 100 gm portion.

This case study points out the risk of masked food allergy to α -amylase.⁴ Indeed, α -amylase is used not only in baked goods and sweets but can also be present in starch, fruits, vegetables, beverages, sugar, honey, and diet foods.⁵ Thus new cases of food allergy to this substance are likely to occur in the future. We suggest that this possibility be

considered systematically in cases of allergy to foods.

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The effects of nebulized recombinant interferon- γ in asthmatic airways

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Recombinant interferon- γ (rIFN- γ) is an immunomodulatory cytokine known to inhibit IgE synthesis and T_{H2} cell proliferation and function.¹ These observations suggest a potential role for rIFN- γ in the treatment of allergic diseases.

In a double-blind, placebo-controlled trial, we have previously shown that patients with atopic dermatitis treated with subcutaneous rIFN- γ demonstrate clinical improvement along with a significant decrease in circulating eosinophil counts compared with placebo-treated control patients.² On the other hand, patients with steroid-dependent asthma treated with subcutaneous rIFN- γ

Abbreviations used

BAL:	Bronchoalveolar lavage
FEV ₁ :	Forced expiratory volume in 1 second
IL:	Interleukin
rIFN- γ :	Recombinant interferon- γ

showed no improvement, although they did have an observed decrease in circulating eosinophil counts.³ One explanation for this might be that the subcutaneous rIFN- γ did not exert a biologic effect in the airways. Indeed, Jaffe et al.⁴ have shown that subcutaneously administered rIFN- γ , given over a 3-day period, does not reach the respiratory epithelial surface. We recently examined the feasibility of administering rIFN- γ by nebulization into the airways of normal subjects and found that rIFN- γ could be delivered safely and effectively over a 12-day interval.⁵ This pilot study was therefore undertaken to evaluate the effects of nebulized rIFN- γ in patients with mild atopic asthma.

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TABLE I. Free IFN- γ levels, expression of IP-10, IL-1 β , and IL-8 gene transcripts and eosinophils in BAL fluid before and after treatment with nebulized rIFN- γ

Patient No.	Free IFN- γ *		IP-10†		IL-1 β †		IL-8†		Eosinophils‡	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	0	54.7	1.02	1.78	2.93	0.79	1.90	0.39	3.25	1.8
2	0	45.9	0.79	1.42	1.55	0.91	0.65	0.39	1.0	0.2
3	0	38.4	0.34	0.50	0.65	0.74	0.44	0.37	0.4	0
4	0	32.0	0.64	1.19	0.39	1.0	0.32	0.18	0.4	0.6
5	0	39.6	0.78	0.99	0.19	0.32	0.15	0.23	1.0	0
Mean $\pm$ SEM	0	42 $\pm$ 4	0.71 $\pm$ 0.11	1.18 $\pm$ 0.21	1.14 $\pm$ 0.50	0.75 $\pm$ 0.12	0.69 $\pm$ 0.31	0.31 $\pm$ 0.04	1.2 $\pm$ 0.5	0.5 $\pm$ 0.3
<i>p</i> Value§	<0.001		0.02		0.46		0.26		0.07	

*Free IFN- γ was measured by ELISA⁵ and expressed in picograms per milliliter.

†mRNA transcripts were measured as described by Martin et al.⁵ with cDNA amplification by polymerase chain reaction. Parallel polymerase chain reactions were performed with either one-twentieth of the cDNA and primers specific for the human γ -actin gene or three-twentieths of the cDNA and primers specific for the human IP-10, IL-1 β , or IL-8 genes. Data are presented as the normalized ratio of either IP-10, IL- β , or IL-8 band density divided by the total band density obtained with γ -actin.

‡Eosinophils are expressed as percentage of BAL white blood cells.

§Determined by Student's *t* test.

METHODS

Subjects

Five nonsmoking adults, aged 22 to 46 years, who fulfilled the criteria for a diagnosis of asthma as defined by the American Thoracic Society⁶ were enrolled in this study. All patients had mild atopic asthma, that is, morning forced expiratory volume in 1 second (FEV₁) greater than 70% of predicted value, requiring no maintenance medications. Each patient had one or more documented positive skin prick test responses to aeroallergens, but none were receiving immunotherapy. Each patient signed an Institutional Review Board-approved informed consent document.

Study design

After baseline spirometry and methacholine challenge, fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) was performed.⁵ BAL fluid analysis for cytology and IFN- γ concentration and measurement of IP-10 transcripts in alveolar macrophages were performed as previously described.⁵ Nebulized rIFN- γ was given in an open-labeled trial according to the following protocol: 50 μ g on days 1, 3, and 5; 250 μ g on days 8, 10, and 12; 500 μ g on days 15, 17, and 19 (total study dose, 2400 μ g). Within 2 hours after the last dose of rIFN- γ , all of the baseline measurements were repeated. In addition to the baseline and posttreatment spirometry, peak expiratory flow rates were measured every morning and evening for 1 week before and during the treatment protocol. A diary, including symptom scores based on severity (0 to 3 for cough, wheezing, chest tightness, and "other") was kept from 7 days before until 18 days after completion of the treatment protocol. Complete blood count and serum biochemistry profiles were done at baseline and on day 19.

Statistics

Comparison between data obtained at baseline and after rIFN- γ treatment for spirometric evaluation, methacholine challenge, and bronchoscopic results were analyzed by Student's two-tailed *t* test for paired values. The sample size had an 83% power to detect a 0.3 L difference in FEV₁ and 93% power to detect a 10% difference in the percent predicted FEV₁. For BAL fluid white blood cells, we had 82% power to detect a difference of 30 cells/ml $\times 10^4$. For peak expiratory flow rate measurements, a mean of the 7-day baseline period and of the 19-day rIFN- γ period was used for each subject, then analyzed by Student's paired *t* test. All analyses are expressed as means $\pm$ SEM. A *p* value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

All patients tolerated the nebulized rIFN- γ well, with only transient mild cough noted during treatment in three patients. The mean clinical symptom scores, provocative dose causing a 20% fall in FEV₁, FEV₁, and morning peak expiratory flow rates were not significantly different from baseline (all *p* > 0.05). No alteration in blood laboratory parameters was noted.

As shown in Table I, the effectiveness of the delivery system was demonstrated by recovery of IFN- γ after, but not before, treatment in BAL fluid (*p* < 0.001). More importantly, nebulized rIFN- γ was effectively delivered to the respiratory epithelium and exerted a biologic effect as measured by upregulation of mRNA for IP-10, an IFN- γ -specific protein induced in activated alveolar macrophages⁴ (Table I).

Treatment with rIFN- γ did not result in any

significant increase in airway inflammation as determined by BAL fluid cytologic evaluation with total white blood cells 11.4 ± 3 cells/ml $\times 10^4$ at baseline versus 14 ± 4 cells/ml $\times 10^4$ after treatment ($p = 0.45$). Cell differential showed no significant changes in percentage of alveolar macrophages ($p = 0.3$), lymphocytes ($p = 0.5$) or neutrophils ($p = 0.15$). Furthermore, gene transcripts of the proinflammatory molecules interleukin (IL)-1 β ($p = 0.46$) and IL-8 ($p = 0.26$) showed no evidence for upregulation after rIFN- γ therapy (Table II). Thus treatment with rIFN- γ did not enhance T_{H1} cell-mediated inflammation.

Of potentially greatest interest, we observed that four of the five patients had a decrease in the percent of BAL eosinophils (Table I). Robinson et al.⁷ have recently shown that improvement in asthma after treatment with a course of prednisolone may be related to the observed decrease in BAL cells expressing mRNA for IL-4 and IL-5 (a T_{H2} pattern) and an increase in cells expressing mRNA for IFN- γ , with decreased number of BAL eosinophils. Because IFN- γ has been shown to inhibit T_{H2} cell function, immunomodulation of IL-4 and IL-5 cytokine production could lead to decreased eosinophil infiltration and activation with a decrease in T_{H2} cell-mediated inflammation. Future studies are needed to examine the potential

role of nebulized rIFN- γ in asthma and other pulmonary diseases.

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Immediate, late, and delayed skin test responses to *Centruroides vittatus* scorpion venom

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As one of 40 species inhabiting the United States, the common Texas striped scorpion, *Centruroides vittatus*, produces a low toxicity venom,¹ which usually causes immediate sharp pain and local swelling after a sting. Venom neurotoxins may also produce skeletal muscle spasms and

Abbreviations used

- LLR: Large local reaction of greater than 24 hours' duration
LPR: Late-phase response

paresthesias, particularly of the sting site, face, and tongue.² Immunologic reactions to envenomation have not been well documented.

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